UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF NEW YORK

Case No. 08 CV 1490-AKH

DREW SCIENTIFIC, INC.,
Plaintiff
vs.
POINTCARE TECHNOLOGIES, INC,.
Defendants.

AFFIDAVIT OF DONALD E. BARRY, JR. IN OPPOSITION TO DREW'S MOTION FOR PRELIMINARY INJUNCTION

I, Donald E. Barry, Jr., declare:

1. I am director of product development at PointCare Technologies, Inc. ("PointCare"). I was PointCare's project manager for the HT project with Drew Scientific, Inc. ("Drew"). I graduated from Colby College in 2003 with a B.A. in Physics and Mathematical Science. I make this Affidavit on personal knowledge, except where specifically stated. I provide this affidavit in opposition to Drew's Motion for Preliminary Injunction.

The HT Project

2. The HT Project was an exciting opportunity for PointCare and Drew to develop and market an automated 22 parameter hematology analyzer with CD4 analysis capability. The basic idea was to take Drew's Excell 22 instrument platform, which was an automated 22 parameter hematology analyzer, and to modify it to perform PointCare's immunogold-based assay and report CD4 results.

3. Unfortunately, despite PointCare's diligent efforts throughout the course of the project to fulfill its contractual obligations, the project failed to produce a viable product. This failure was, in my estimation, due to poor engineering choices by Drew, failure to properly and

efficiently test hardware modules by Drew, and a lack of commitment by Drew staff to complete the project in a reasonable time frame. Indeed, Drew was unable to complete project tasks it was responsible for (in particular, hardware engineering tasks) even though it continued working on the project for approximately one year past the final completion date set forth in the Agreement.

Feasibility of the HT Project

4. Before entering into the Agreement to undertake the HT project, PointCare and Drew jointly conducted a feasibility analysis. As part of the feasibility analysis, PointCare and Drew conducted testing on a cooperative basis from January through March 2006. I personally traveled with my superior, Dr. Peter Hansen, to Drew's Dallas facility to participate in testing whether the PointCare CD4 immunogold assay could work on Drew's existing Excell platform with only minor, straightforward modifications. As a result of the testing, Dr. Hansen and I, along with Roger Bourree of Drew, were able to demonstrate that, with only minor modifications to the Excell's optics, Drew's Excell 22 instrument could provide CD4 results using PointCare's assay. All three of us had considered the optics the biggest potential hurdle to the proposed development of an HT machine. Having shown that this would not be an obstacle, we (PointCare and Drew) agreed to proceed with additional feasibility testing and development of selected modules.

5. Dr. Hansen and I then returned to PointCare where we developed a project plan for the HT and conducted additional feasibility and development work. At the same time, Mr. Bourree, who championed the idea that this was a viable project, was to continue feasibility testing on Drew's end and begin work developing methods for fluid delivery and handling, as well as instrument control software.

6. After the additional feasibility work, Dr. Hansen and I reached the conclusion that the proposed HT project was a "low risk" project. We reached this conclusion based on the successful testing of the PointCare assay with the Excell device with modified optics as well as our knowledge of pre-existing technologies available in the market that carried out the sample preparation and fluid delivery functions needed in the HT. More specifically, PointCare had already developed an instrument (the AuRICA) with automated mixing and delivery of its gold reagent, and Beckman Coulter, a well known medical device manufacturer, sold a machine (the TQ Prep) that had automated mixing and delivery of the same lyse used by PointCare with its assay. Furthermore, PointCare had already proven that the CD4 gold assay was viable, robust, and highly marketable. Drew had only to combine the fluid delivery and handling technologies of the TQPrep and the gold preparation and delivery system of the AuRICA with Drew's existing Excell 22 and add the necessary instrument control software for the new hardware modules. Drew, however, insisted on using alternative fluid delivery and handling techniques in the HT. Since Drew was responsible for the hardware modifications to its platform and the engineering choices to implement those, PointCare respectfully accepted Drew's engineering decisions and provided support wherever and whenever needed. Ultimately, Drew failed to successfully implement its engineering choices in the HT and failed by a long shot to meet its contractual obligations to modify its Excell 22 (later replaced by the Excell 2280) pursuant to the contract deadlines.

Drew's Inability to Overcome Obstacles Created by Its Own Engineering Choices on the HT Project

7. During the course of the HT project, one of the hurdles that Drew needed to overcome was the inability of the optical sensor selected by Drew, which was part of the fluid delivery module that Drew had designed, to reliably and repeatedly detect the presence of

PointCare's gold reagent. The fluid delivery modules contained in the two systems that PointCare has successfully used in conjunction with its immunogold-based assay (the AuRICA System and the NP System) are "pipette-based" systems. This means that they use an automated needle and syringe to dispense the gold reagent, rather than relying on a system of pumps and optical sensors, as Drew chose to employ. Early in the project (approximately June 2006), I warned Drew engineering that, although we had never attempted to use an optical sensor as part of a method for gold delivery, during our work on the AuRICA System we had observed the gold "stain" the channels of an acrylic block used in the instrument. This information was captured in a document that I am informed was recently produced (April 18) by Drew to PointCare's counsel which I understand contains hand written notes of Drew's HT project leader, Gary Young, dated June 13, 2006. See Exhibit 1 attached hereto (document DR00074904 and e-mail from Drew's counsel to PointCare's counsel conveying the document; the relevant portion of the document text has been circled). I then cautioned Drew against using acrylic in connection with the gold reagent because of a chemical incompatibility. It was at this point that I also suggested that Drew could try polycarbonate because there is no chemical incompatibility between the gold and polycarbonate. Drew engineering did try to use the polycarbonate, but failed to effectively smooth the material, causing the gold to physically adhere to the surface of the polycarbonate. It is important to note that this was not a chemical reaction but a surface effect that was a result of the manufacturing process chosen by Drew. Indeed, PointCare never specified or demanded that Drew utilize specific materials or employ specific manufacturing processes for the HT hardware, nor did PointCare have the authority to do so. Indeed, PointCare did not know the capabilities and limitations of Drew's optical sensor, nor was PointCare aware of Drew's abilities and limitations to machine, smooth, and polish different materials. I did warn Drew engineering that

if material surfaces contacting the gold were not properly cleaned and polished, there existed a possibility the surface effects of the materials could grab the gold and that that could affect the functioning of Drew's selected optical sensors. However, without knowledge of a smoothness rating for Drew's machining capabilities, nor the details of the sensor selected by Drew, I was unable to provide any further information on the topic other than chemical compatibility. Since Drew bore the responsibilities to modify the Excell 22 platform to accommodate PointCare's CD4 immunogold assay, PointCare relied on Drew's engineering capabilities to properly design, test, and manufacture an effective fluid delivery system as was Drew's obligation under the Agreement.

8. The Drew brief (p. 15) falsely states that, "Because the NP was not a high-volume machine, it did not experience the same gold adherence problems as the HT." The intended higher volume capability of the HT as compared to the NP machine had no bearing on the "gold adherence problem" in the HT. In actuality, the NP does not use any optical sensors to detect the presence of gold and therefore, there is no issue to address. The NP uses pre-existing pipette-based technologies that were proven to work on the manufacturer's base system. In addition, the HT prototype delivered by Drew could not run more than a handful of samples before the optical sensor malfunctioned. Since the HT system was intended to analyze more than one hundred samples per day, a failure after only a few samples does not indicate that the expected higher volume use is what caused the "gold adherence problem," nor does it make a viable product.

9. By March 2007, the Drew engineering team was aware of the difficulties presented by their selection of optical sensors for use with the HT System and failed to address the issues in an appropriate and timely manner. Drew engineering had to solve one of two issues with the gold delivery module: either find a way to prevent gold buildup or find an alternative to

the optical sensors not effected by the gold buildup. Due to Drew's reluctance to change its original design and replace the optical sensors, PointCare devoted additional efforts and resources to help Drew try to overcome this obstacle. In March 2007, the project timeline, which was already months behind schedule, was in serious danger of slipping even further. In an effort to help move the project along, I immediately asked for help from some outside sources to find a solution to the gold adherence problem. Although there were certain materials (such as polyethylene, polypropylene, and Delrin) that could improve the effective transfer of the gold reagent and potentially eliminate the adherence problem, none would meet the additional criteria imposed by Drew (i.e., that it had to be both machineable and clear in color). On March 17, 2007, I notified Drew engineering that we were "stumped" to find a material that is both "machineable" and "transparent" to permit their selected optical sensors to work on a reliable and repeatable basis. I then suggested that they use an ultrasonic sensor, which would have no dependence on the optical properties of the material used. Gary Young, Drew's Project Manager for the HT, refused to use an ultrasonic sensor when I suggested it to him in March 2007 because he was more comfortable using optical sensors. Although I expressed my concern verbally and in writing (see Exhibit 2 attached hereto, DR00023621-22, which is a copy of an e-mail I sent to Gary Young) that Drew's optical detection method was inherently flawed for this application, Mr. Young persisted in his attempts to make the optical sensors work. Drew engineering ultimately came to the realization during the summer of 2007 that their insistence on using an optical sensor was not practical and an ultrasonic sensor was a better design choice. It then took Drew an unreasonable amount of time (several more months) to implement the change to the ultrasonic sensor.

10. The assertion in the Drew brief (p. 12) that I admitted fault and claimed responsibility for the problems with the Drew optical sensor is a gross overstatement of what I said. What I actually said was: "I feel bad and somewhat responsible that we are having trouble with that optical sensor." The purpose of this email was to commiserate with the Drew engineering staff as it was apparent to me that they were losing focus and losing confidence in their ability to get the project done. Gary Young, at one point, confided in me that he was in fear of losing his job based on poor performance on the HT project. Various PointCare staff sent inspirational and supportive emails to their colleagues at Drew in that time frame in an effort to boost the morale of the Drew engineering group which appeared laden with distress during this critical period of development.

11. Although the Drew team was having difficulties with the project, PointCare was still optimistic and actively trying to help bring the project to successful completion. However, starting around early summer 2007, it appeared that the Drew team was giving up on the project as they began ignoring my requests for status updates. Although I had sent large volumes of very expensive gold reagent for testing as early as summer 2006, Drew engineering failed to properly test their selected modules prior to integration into the HT System in early 2007. During my visit to Drew in January 2007, they had actually "lost" the container of gold in their laboratory. It was obvious to me, based on my interaction with Mr. Young and others at Drew, that throughout the life of the HT project Drew had other priorities, such as other projects, that prevented them from applying the needed attention and testing to the modules for the HT project within the agreed upon timeframe. E-mails from Gary Young to me illustrate this point. See Exhibit 3 (DR00019932 and PointCare 000088) hereto which are true and accurate copies of e-mails sent and received by me. The mismanagement of design tasks by Drew caused them to

take shortcuts and fail to properly test modules, as required by standard engineering practices which caused additional problems and delays on the project. Again, e-mails from Gary Young to me illustrate this point. See Exhibit 4 (DR00023777 and PointCare 000094) hereto which are true and accurate copies of e-mails sent and received by me.

12. Drew has repeatedly fallaciously stated in its claims that it was unable to complete hardware development because PointCare had not delivered software. The two main design obstacles that Drew was unable to solve were independent of any software that PointCare was developing. In fact, at no time was Drew able to produce any data from an automated HT instrument that could be accurately analyzed by PointCare software. Drew was responsible for writing all of the hardware control software. This software alone is enough to test the movement and detection of the gold reagent using their sensor of choice, whether it be optical or acoustical (ultrasonic). Standard engineering practice is to create a test fixture that can perform repeated functions to get an estimate of failure time. For example, Drew could use their hardware control software without input from PointCare to determine that their selection of an optical sensor is not appropriate for this technology due to the small number of runs between failures. Drew admits that they did not finish the design of the gold sensor until November 2007 (Drew brief p. 16), well more than a year after the agreed upon date set forth in the contract timeline for development and integration of all hardware modules.

13. The other main design obstacle that Drew encountered was how to develop an effective method for mixing the PointCare reagents. PointCare had delivered to Drew a proven mixing method (vortex mixing) for the CD4Sure assay, which was spelled out specifically in the Agreement (See Agreement, Annex 1, Attachment 3). The specific sequence and type of mixing was again reinforced during a meeting between the two companies in June 2006 (see Exhibit 1

hereto). Drew, based on its experience, chose to use a different mixing method (paddle mixing, as opposed to vortex mixing) to handle the PointCare reagents—a risk that Drew bore on its own. The testing of the mixing task only required the hardware control software, developed by Drew, and qualitative analysis to determine a satisfactory end point. I supplied Drew engineering with results obtained by PointCare's vortex mixing method so that Drew had a firm end point as a reference. It was clear to me from data I received from George Chappell in November 2007 that Drew had still not completed the mixing sequence that is clearly outlined in the Agreement (see Agreement, Annex 1, Attachment 3). To this date, I have not seen any results from Drew that demonstrate that they have completed the mixing module and met the requirements outlined in the Agreement. Again, this failure was mainly due to the fact that Drew insisted on using paddle mixing, which it preferred, instead of other known methods of success outlined by PointCare (see Agreement, Annex 1, Attachment 3).

14. In Drew's brief (p. 15), Drew suggests PointCare obstructed Drew's efforts to solve the gold adherence problem. This is false. During the summer of 2007, I received notice that a member of the Drew engineering team had contacted one of our gold vendors. Lacking further information, I called the vendor to inquire about the nature of the conversation. The intent of my call was simply to inform the vendor that although Drew and PointCare were working on a project together, Drew was not privy to the same trade secrets that PointCare shared with the vendor. Nevertheless, I did instruct the vendor to provide Drew with any information necessary to help Drew solve any of the problems it was having with the optical sensors as that was a critical design flaw that had brought our project to a standstill.

Response to Specific Arguments Made by Drew in Its Brief

15. On page 12 of the Drew brief, Drew appears to argue that my use of the word "assumption" in a document dated February 2007 somehow suggests PointCare had not done a legitimate feasibility analysis supporting its conclusion that the Excell 22 could be modified to work with PointCare's assay. Drew simply misunderstands the meaning of the word "assumption" in the context of that document. The term "assumption" as I used that term in the cited document simply denotes the logical "premise" for the HT project. The term "assumption" is a term-of-art commonly used in regulatory documentation. It means that the proof of the statement may be found in other traceable documents, but not in this one. In this case, the "proof" is listed as "conclusions" that are identified in my feasibility report which firmly states that: "The Excell 22 system can be modified to accommodate a CD4 immunogold assay." See Exhibit 5 attached hereto (Exhibit 1 to Barry Dep.), which is a feasibility report I wrote. This statement is further supported by PointCare's study in Barbados conducted in October 2006, which showed that PointCare's CD4 immunogold assay can successfully produce patient results on a modified Excell 22. So, in this case, the "assumption" was proven to be a true one.

16. The Drew brief at page 11 falsely states that PointCare "[made] use of an existing, unmodified Excell machine." This would have been impossible as without the optics modification, provided by Drew, to include a different angle of light scatter, no CD4 results could have been obtained. The machine used had been modified by Drew to include a prototype optics assembly capable of CD4 analysis. Drew only needed to implement automated fluid delivery and handling hardware and controls to complete the system.

Conclusion

17. As PointCare's project manager, I had personal and professional incentives to see the project come to a prompt and successful completion. Contrary to Drew's claim that I intentionally sabotaged their efforts to solve their engineering issues (Drew brief p. 2), I went out of my way to help try to solve these issues to bring a successful product to market for both parties. From the beginning of the project in January 2006 until the termination of the agreement, I worked diligently on the HT project. I always made myself available to Drew to provide assistance when needed and continued to provide prompt, detailed, and thoughtful comments throughout the life of the project. Unfortunately, despite my input, there were times that Drew either chose to ignore my suggestions or failed to assimilate knowledge. As project manager for both the NP and the HT projects, I responsibly allocated PointCare resources to provide attention to both projects without preference for one over the other. PointCare staff typically worked long days and made extended trips to Drew to help address Drew's hardware design issues on the HT project. There were times when, due to Drew's failure to produce functioning hardware modules, PointCare could do little or no work to further the HT project. Specifically, this happened in the summer of 2007 when PointCare was stuck with an unusable HT instrument and was waiting for Drew to solve its hardware design flaws. The reality is the HT project failed because Drew was unable to fulfill its engineering obligations despite PointCare's continuing support.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct. Executed this 25 day of April, 2008, in Marlborough, Massachusetts.

Donald E. Barry, Jr.

01239005

EXHIBIT 1

6-13-06

(2)

SAMPLE PREP.

45 WHOLE BLOOD ± 10%

45 ACC ± 30%.

20 GOLD REAGENT +6 -30%

SEQ. IS NOT CRITICAL.

THIS MIX STEP IS NOT CRITICAL 3-5 SEC. VORTEX MIXING.

PADDLE MIXING.

37°C REACTION CHAMBER TEMP. CUVETTE

3 MIN. MAX INCUBATION BEFORE LYSING.
(PROBABLE TIME 45 SEC - 60 SEC)

2 PASS TEST (1ST NORM. TEST 2ND CD4- TEST)
RUN NORM. TEST DURING INCUBATION

ACIDIC CD-4 LYSE 300µL ± 10%.

MIXING & TIMING CRITICAL. 10 SEC MIX

STEPPER MOT. SPEED OF MIX CRITICAL. MAY REQ. RAMPING.

ADD QUINCH 135µL ± 10% 10 SEC.

NEUTRAL PH & OSMOLALITY.

GOLD REAGENT

GOLD REAGENT TURNS ACRYLIC BROWN AFTER ONE MONTH.
NO AFFECT ON TYGON OR DELRIN.

REAGENT CONTACT TIMES (SHORT) MAKE REACTION MINIMAL.

Michael P. Twohig

From: Dellaportas, John [Dellajo@duanemorris.com]
Sent: Friday, April 18, 2008 6:26 PM
To: Michael P. Twohig
Cc: Andrew F. Caplan; Damiano, Brian J.; Costantini, Anthony J.
Subject: Drew v. Pointcare

Attachments: DR007.zip

DR007.zip (2 MB)

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Warning: Your file, DR007.zip, contains more than 32 files after decompression and cannot be scanned.
**********-**********

Michael:

Further to your request at Mr. Young's deposition, attached are certain additional responsive materials from Mr. Young's office. We believe them to be largely duplicative of prior productions, but in an abundance of caution are producing them all. We will send you a CD-Rom with the same documents on Monday. Please note that one of the documents is labeled "Attorneys' Eyes Only." Thanks.

John Dellaportas
Duane Morris LLP
1540 Broadway
New York, NY 10168
Tel.: (212) 692-1012
Fax: (212) 202-4866

EXHIBIT 2

From: Don Barry
Sent: 3/16/2007 6:40:27 PM
To: Gary Young
CC:
Subject: RE:

Hi Gary,

Nobody will be at PointCare tomorrow, so Saturday delivery will not be necessary. I was thinking of coming in, but we are getting hit with a blizzard tonight. I would like to get started on Monday morning, if possible.

Did you decide on trying a glass tube for the gold measurement? I am very concerned that even if we replace the board, we will still have an issue with the gold build-up in the channel with the current design. Did you guys come up with a different solution?

Thanks,

Don

From: Gary Young [mailto:gyoung@mwi-danam.com]
Sent: Thursday, March 15, 2007 4:53 PM
To: Don Barry
Subject: RE:

Don,

The board and sensors will not ship today. We will ship them tomorrow with overnight delivery.

Gary

From: Don Barry [mailto:debarry@pointcare.net]
Sent: Thursday, March 15, 2007 3:41 PM
To: Gary Young
Subject:

Hi Gary,

Does it look like you will be able to ship another board and sensors today?

Thanks,

Don

EXHIBIT 3

From: debarry@pointcaretechnologies.com
Sent: 7/28/2006 10:07:21 PM
To: Gary Young
CC:
Subject: Re: Further delay on shipping the optic head w/ integrator

Hi Gary,

I understand your small staff and limited time for two concurrent projects. Thank you for continuing to keep me informed with the project-your and your team's efforts and commitment continues to be greatly appreciated.

Have you been able to run any samples with the integrator? Any initial observations?

Don
Sent from my BlackBerry wireless handheld.

-----Original Message-----
From: "Gary Young"
Date: Fri, 28 Jul 2006 17:45:22
To:
Cc:"Andrew Kenney"
Subject: Further delay on shipping the optic head w/ integrator

Don,

Due to EMC testing issues with the 2280, and our small staff to address these issues, the optic head delivery will be delayed again.

The new integrator PCB has been installed and run. The unit we attempted to test it on is the #1 engineering prototype and it has reliability problems that are keeping us from completing the testing. Fortunately, we have one other [pre-production] unit here we can install it in. That is planned for Monday.

If all goes well, we can ship the head Tuesday.

Please see the attached photos.

Regards,

Gary Young
Drew Scientific

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Eric Newman

From: Don Barry
Sent: Wednesday, October 31, 2007 4:58 PM
To: Eric Newman
Subject: FW: Emailing: HTS Version 4.2 Plan.pln

-----Original Message-----
From: Gary Young [mailto:gyoung@mwi-danam.com]
Sent: Wednesday, September 06, 2006 4:51 PM
To: Don Barry
Subject: RE: Emailing: HTS Version 4.2 Plan.pln

Don,

Thanks for the plan. I've been tied up the last two days with the TUV safety inspector trying to get the safety certification for our DS5 so we can sell it in China.

I'll look it over, make my changes and send it back.

Gary

-----Original Message-----
From: Don Barry [mailto:debarry@pointcare.net]
Sent: Wednesday, September 06, 2006 2:53 PM
To: Gary Young
Subject: Emailing: HTS Version 4.2 Plan.pln

<<HTS Version 4.2 Plan.pln>>
Hi Gary,

Please see the attached revised project plan. I had to guess at some of the dates, so pleasee feel free to make nay changes.

I'm not sure exactly how you would like me to measure the PMT positioning. If you can give me some description or a part drawing, maybe I can give you something useful.

Don

EXHIBIT 4

From: Gary Young
Sent: 4/10/2007 11:32:40 PM
To: dbarry@pointcare.net
CC: Andrew Kenney; George Chappell
Subject: Optichead Flowcell mount problem

Don,

After several hours of attempting to align the optichead we've concluded the problem is with the quartz flowcell glued to the PEEK mount.

The spare we had (for just this problem) had the same problem also.

We have removed the optichead from the second unit we are building for you and are checking the alignment on it.

Due to the time spent in determining the problem, we did not get the optichead shipped today. It will ship Wednesday with a scheduled delivery for Thursday

This will delay us shipping this next unit by at least one week. This delay is due to the slow cure time of the adhesive used to bond the quartz flowcell to the PEEK.

If you have any questions, please call me.

Regards,

Gary Young
Drew Scientific, Inc.
214-210-4922

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Eric Newman

From: Don Barry
Sent: Wednesday, October 31, 2007 5:00 PM
To: Eric Newman
Subject: FW: Repair of BAR-8202-001 PCB

From: Gary Young [mailto:gyoung@mwi-danam.com]
Sent: Thursday, August 10, 2006 10:48 AM
To: Don Barry
Subject: RE: Repair of BAR-8202-001 PCB.

Don,
I can only apologize for us not getting it right the first time. Based on my limited knowledge of electronics, George's summary sounded to me like he failed to test it adequately before we shipped it.

Gary Young

From: Don Barry [mailto:debarry@pointcare.net]
Sent: Thursday, August 10, 2006 9:42 AM
To: Gary Young
Subject: RE: Repair of BAR-8202-001 PCB.

Hi Gary,

I just received the package. Thanks for the quick turnaround and thorough analysis.

Don

From: Gary Young [mailto:gyoung@mwi-danam.com]
Sent: Thursday, August 10, 2006 10:01 AM
To: Don Barry
Subject: FW: Repair of BAR-8202-001 PCB.

From: George Chappell
Sent: Wednesday, August 09, 2006 6:39 PM
To: Don Barry (debarry@pointcare.net)
Cc: Gary Young
Subject: Repair of BAR-8202-001 PCB.

Here is a summery of what I did and a few tips on the setup procedure. I believe Gary had the board shipped this afternoon , but I don't know if he sent it overnight or second day.

GDC

11/1/2007

EXHIBIT 5

From: Peter Hansen
To: Roger Bourree; rbourree@MWI-DANAM.COM
Subject: Don's Report
Date: 4/5/2006 6:27:51 PM

Attachment N1: HT-0001 System Mod Feasibility.doc

Title of Project: High Throughput System for Developing World Market

Date: March 27, 2006

Author: Don Barry

Approvals:

APPROVALS				
name	Signature	date	Title	Document Approval Function
Don Barry			Scientist/Engineer	Originator/Project Manager
Romiya Glover			Scientist	Technical Review
Maurice Doire			Director, QA/QC	QA/QC

Abstract

The Excell 22 (Drew Scientific) has been identified as an instrument that can be adapted to accommodate a CD4 immunogold assay. This system has the potential for becoming a high throughput analyzer for approximately 100-150 hematology plus CD4 samples per day.

The PointCare lysing system (Erythrolyse/Stabilyse) has proven to be more effective in CD4 cluster presentation than the Drew lyse. The Drew lyse will remain onboard for the gold-free 5-part leukocyte differential. The Drew paddle mixers can be used to lyse samples for CD4 analysis with the Erythrolyse. Further optimization is to be done in the Excell 22 mixing chamber.

The Excell 22 optics has been modified to accommodate the CD4 immunogold assay. A new "Right-Angle Scatter" (RAS) detector has been added to the optical assembly for improved CD4 analysis over the Excell 22 "Super-Wide Angle" (SWA) Detector. A black matte finish has been applied to the interior of the optical assembly to reduce stray light. The Excell 22 does not currently have any integration on the detectors, but this may have to be implemented for enhanced CD4 cluster presentation.

Fluid delivery modules will have to be added to the Excell 22 to accommodate the addition reagents required for the CD4 immunogold assay. A gold reagent and accelerant delivery module as well as delivery for the Erythrolyse and Stabilyse will have to be implemented. There is an auto-sampler that PointCare would like to use for all systems being sold for CD4 analysis. This would allow the system to be operated for 30 samples without interruption.

Modifications to the Excell 22 analytical software will have to be made for CD4 cluster recognition as well as flagging criteria. The Excell 22 user interface will also have to be modified for CD4 analysis of patients and controls.

Some of the components of the Excell 22 are open and susceptible to dust and particulate collection. These components will have to be examined for the environment that PointCare plans to place these instruments. Internal control points for temperature, humidity, and door and cover sensors will also have to be addressed.

1. **Purpose**

   a. Drew Scientific currently sells an instrument by the name "Excell 22" that has the potential to utilize a CD4 gold assay. The purpose of this investigation is to examine the possibility of adapting the Excell 22 to analyze a CD4 gold assay and determine the necessary hardware modification to do so.

2. **References and Attachments**

   b. PointCare Lab Notebook PCT-1035, pages 1-2, 20-32

   c. PointCare Lab Notebook PCT-1040, pages 16-19

   d. Drew Scientific Visit Report 010506

   e. Drew Scientific Visit Report 021006

   f. Bikoue, A., et al. *Quantitative Analysis of Leukocyte Membrane Antigen Expression: Normal Adult Values.* Cytometry. Vol. 26: pages 137-147. 1996.

3. **Test Results**

   g. **Description and Status of Testing:**

Task #	Test Task	Critical Element	Schedule	Responsibility
1.	Decide between Drew RBC lysing reagent and PointCare RBC lysing reagent	Lysability and CD4 cluster separation	2/10/06	D. Barry
2.	Evaluate Excell 22 paddle mixers	Lysability and CD4 cluster separation	3/31/06	D. Barry
3.	Evaluate Excell 22 optics as platform for PointCare immunogold assay	CD4 cluster separation	3/31/06	D. Barry/ P. Hansen
4.	Evaluate Excell 22 data handling electronics and sample handling electronics	Flexibility necessary for CD4 assay	2/10/06	D. Barry
5.	Determine design options for immunogold dispensing	Small volume (~10 uL) fluid delivery	3/31/06	D. Barry
6.	Determine gates and regions for analytical software development	New gates for CD4 lymphocytes	3/31/06	D. Barry
7.	Evaluate Excell 22 user interface	CD4 analysis capability	3/31/06	D. Barry

8.	Determine dust-sensitive components of Excell 22	Particulate interference	2/10/06	D. Barry
9.	Determine compatibility of auto-sampler	Throughput expected, number of samples held, and sample volume delivered	2/10/06	D. Barry
10.	Evaluate internal control points in Excell 22	Complete hardware and assay control points	3/31/06	D. Barry

h. Significant Test Results

i. Both the Drew five-part differential lysing reagent and the PointCare lysing reagent (Erythrolyse II) are acceptable for red cell lysis. The PointCare lysing reagent did however produce greater CD4 cluster separation than the Drew lyse (figure 1). Please see below a legend for Excell 22 parameter numbers:

Parameter Number	Description	Angle
1	Low Angle Scatter (LAS)	~3°
2	Extinction (EXT)	0°
3	Wide Angle Scatter (WAS)	~8°
4	Super Wide Angle Scatter (SWA)/ Right Angle Scatter (RAS)	~30° - 45° for SWA, 65° - 115° for RAS

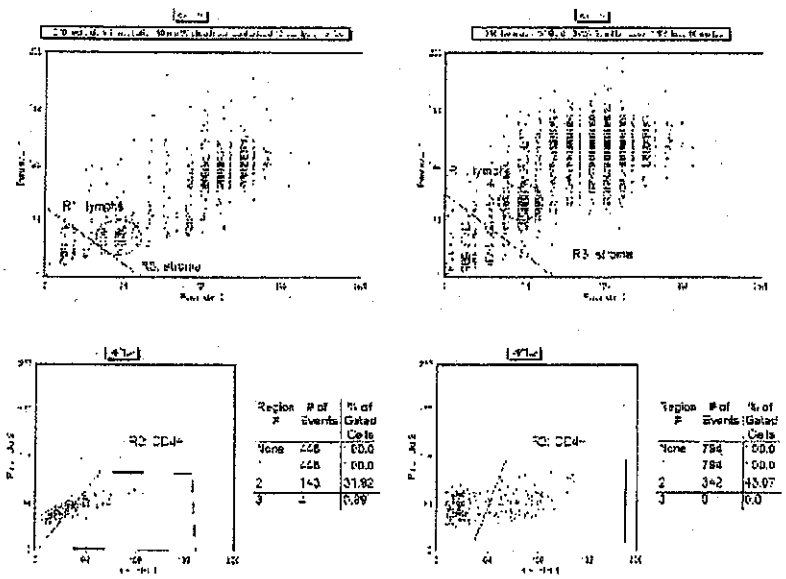


Figure 1: The plots above demonstrate that both the Drew lyse (left) and the PointCare lyse (right) can produce clean RBC lysis, but only the PointCare lyse presents a clear CD4 cluster.

ii. The paddle mixer and mixing sequence that exists in the Excell 22 did not provide sufficient mixing to completely lyse the red cells and present CD4 cluster separation similar to off line vortex mixing. A breadboard of the paddle mixer with a stepper motor to control mix speeds and times was developed to evaluate if the paddle mixer could be used with a different sequence.

The vortex sequence of 3 seconds mix with blood, gold, and diluent, then add lyse and vortex for 8 seconds, then add quench and vortex for 10 seconds is considered to be the standard to compare to [PCT-1035: 1-2]. All vortexing is done at 1700 rpm. The standard volumes are 50 μL whole blood, 50 μL diluent (PBS with 0.1% polybrene), 20 μL gold, 300 μL Erythrolyse II, and 133 μL Stabilyse [PCT-1035: 1-2]. An example of this sequence using an AuRICA for the analysis portion can be seen in figure 2.

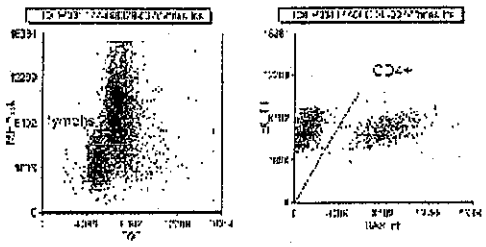
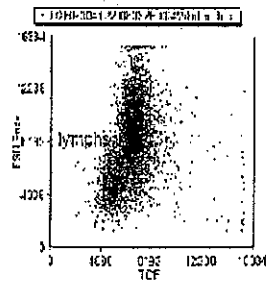
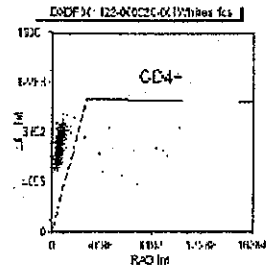


Figure 2: The plot above shows the Erythrolyse when vortexing is used to lyse the sample.

When the same sequence is used with the paddle mixer, a clean leukocyte differential can be seen when no gold or diluent are used (figure 3). When gold is added, the CD4 cluster is present, but there are some unlysed RBCs present (figure 3).


lymphs
TOF


CD4+
RAD Int

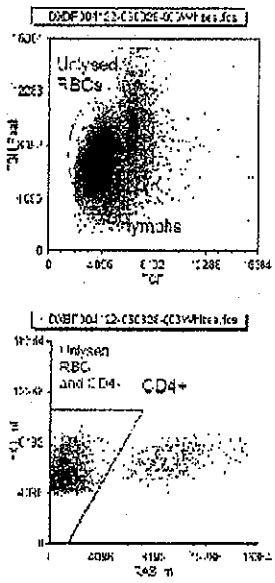
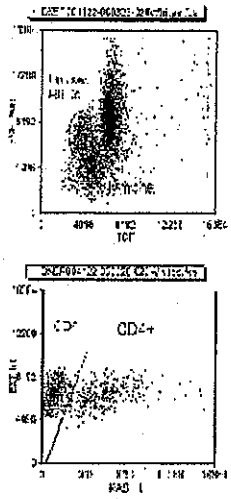
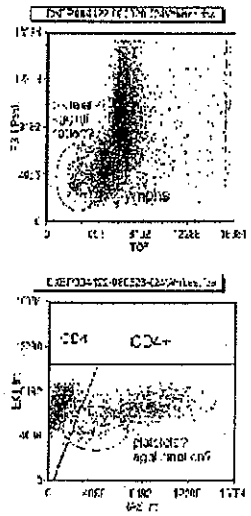


Figure 3: The left plot shows that when the paddle mixer is used with no gold or diluent, a clean WBC differential can be seen. The right plots shows that with the same sequence but with gold added, the CD4 cluster is present, but unlysed RBCs are present as well.

It is still possible to use the paddle mixer to obtain both an easily discernable CD4 cluster and a clean leukocyte differential. Some options for modification of the lyse sequence include lyse and quench volume adjustment, lysing time adjustment, and lyse mixing speed. Complete results from testing using these sequences can be found in notebook PCT1035: 20-32.


CD4+


CD4
CD4+

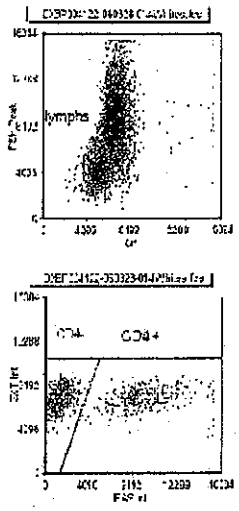


Figure 4: The left plot shows that when the lyse volume is increased from 300 to 400 µL, the RBCs are decreased. The center plot shows that when the mix speed is increased from 1700 to 3400 RPM, the RBCs disappear, but it looks like there may be some platelet aggregates or possibly agglutination of leukocyte fragments or protein. The right plot shows that when the lyse mix time is extended to 12 seconds, but the mix speed and volumes are the same, the RBCs tend to disappear.

Optimization of the paddle mixer should be done using the Drew Excell 22 mixing chamber. The geometry and material of the chamber is different than that of a 12mm polypropylene culture tube and the mixing may be slightly different. It may be necessary to modify the Excell 22 mixing chamber so that no reagent is lost through the bottom of the cuvette.

iii. The existing optics in the Excell 22 had to be modified to accommodate the CD4 assay. The Excell 22 optics currently has a " super-wide angle" detector that has a mask to detect eosinophils at 30° to 45°

(figure 5). To be able to see the CD4+ cells separate from the CD4-, the mask had to be removed to allow an angle of 30° to ~90°.

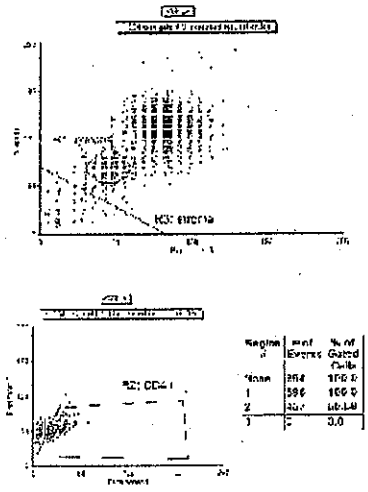


Figure 5: The plot above shows PointCare lyse with Excell 22 optics as manufactured today with a mask on the super-wide angle detector.

In order to see an improved CD4 cluster presentation, the gain for the super-wide scatter detector was nearly doubled. This did improve the visibility of the CD4 cluster, but also added noise. The scatter gain was then brought down to about 1.5 times the original gain and similar results were seen with slightly less signal (figure 6).

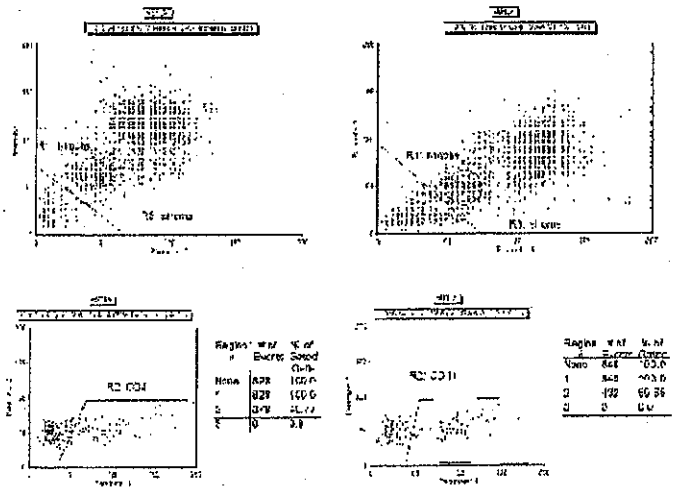


Figure 6: The left plot shows the mask removed and the gain doubled on the super-wide angle detector. The right plot shows a gain of 1.5 times the original. In both cases, an increased noise is seen.

The Excell 22 laser normally runs at about 2 mW. When the laser power was increased from approximately 3 mW to 4 mW (1.9V), cluster definition was improved without a significant increase in noise (figure 7). It may be necessary to use a higher powered laser to easily define a CD4 cluster. PointCare currently uses a laser running at 8 mW to visualize a CD4 cluster. The Drew system does have a PMT that may prevent the need for a higher powered laser. The beam profile in the Excell 22 optics is 200 µm wide by 20-25 µm tall. This appears to be acceptable for sizing the cells as well as producing enough signal for all detectors.

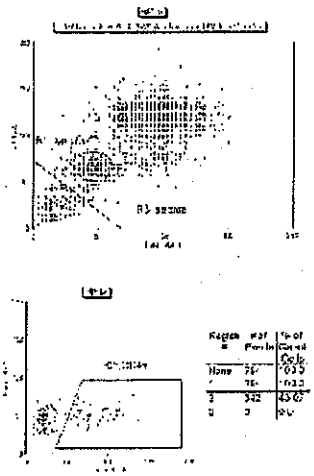


Figure 7: The above plot shows an increased CD4 separation by increasing the laser power without increasing the gains.

The PMT for the super-wide angle has a light collection lens. We removed this lens to see if we could eliminate an extra alignment step in manufacturing, as well as the need for an extra part (figure 8). It does appear that even with the lens removed, a CD4 cluster can be seen. Just to note, a different gold lot was used that may account for differences in CD4 separation from previous analysis.

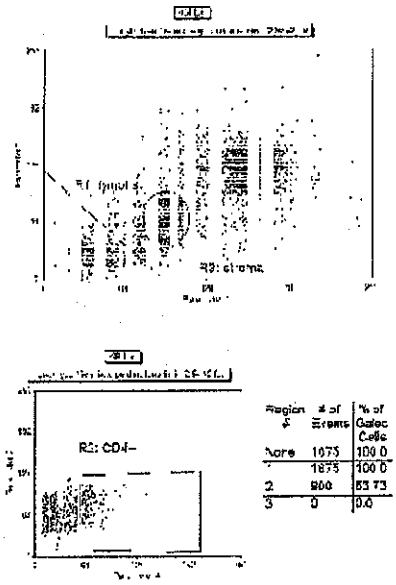


Figure 8: The above plot shows the CD4 cluster without a light collection lens.

There was a modified optical assembly at Drew with the internal walls of the optics covered with a matte black finish to reduce stray light. This increased the signal of the clusters and should help us in locating the CD4 clusters (figure 9).

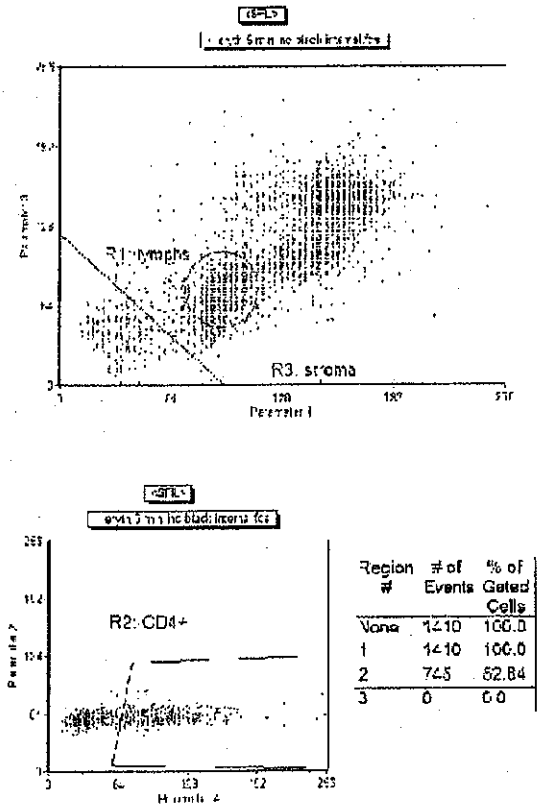


Figure 9: The above plot shows the CD4 cluster with a blackened interior and without a light collection lens.

In order to analyze both eosinophils (by use of the mask) and the CD4 cluster, an additional detector (PMT) was added to the other side of the Excell 22 optics. It is possible to identify eosinophils without the mask (figure 10), but the mask is an enhancement. The interior of the optical assembly does have a black finish to reduce stray light. The additional detector does have a light collection lens but there is no mask. The lens may not be necessary for the RAS detector, but more testing paying close attention to noise will have to be done. This detector is repositioned to be centered at 90° with a range at approximately 65° to 115°. A CD4 cluster could be easily seen using this optical assembly (figure 11) when the laser is

run at 1.5 mW. Although it is difficult to determine if increasing the laser power improved cluster definition in the case, previous testing has shown that this may be an improvement. It may be necessary to further increase the laser power for larger CD4 cluster separation.

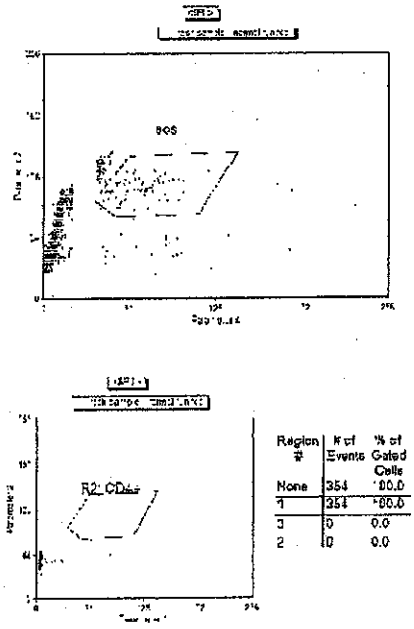


Figure 10: The plot above shows that when the Drew 5-part differential lyse is used with a sample without gold, the eosinophils are easily distinguished, even without the mask.

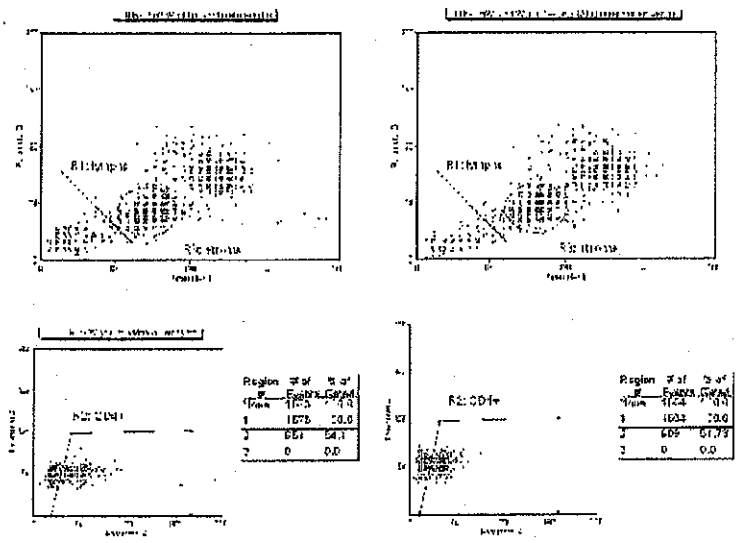
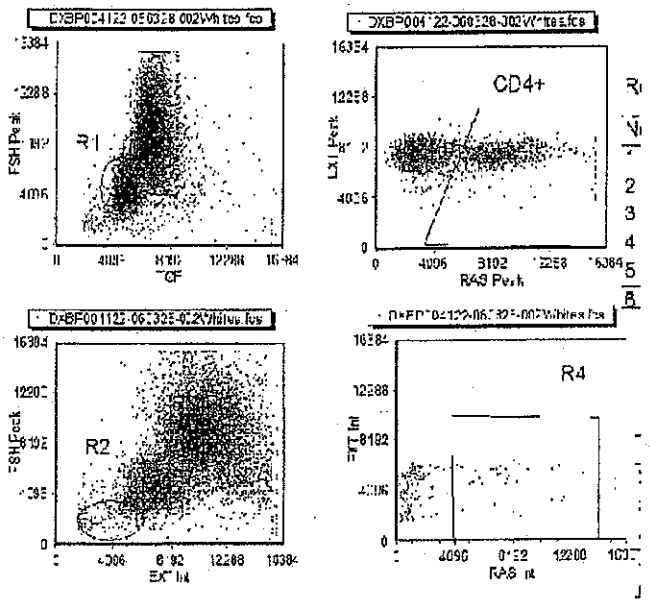
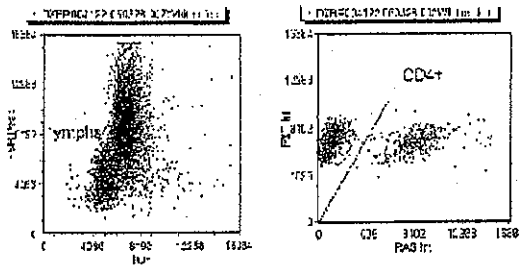


Figure 11: The left plot shows that when using the additional right-angle scatter (RAS) detector without a mask, a CD4 cluster can be seen. The right plot shows an increased laser power from 1.5 to 2 mW.

The current PointCare electro-optics design on the AuRICA System uses a higher power (8mW) laser and has analog integration on the RAS preamp. A slight improvement to the CD4 cluster presentation can be seen with higher laser power, but the majority of the enhancement is done by the integration (figure 12). For this reason, it may be necessary to add integration to the Drew optics for optimal cluster presentation.


CD4+
R2
R4


CD4+

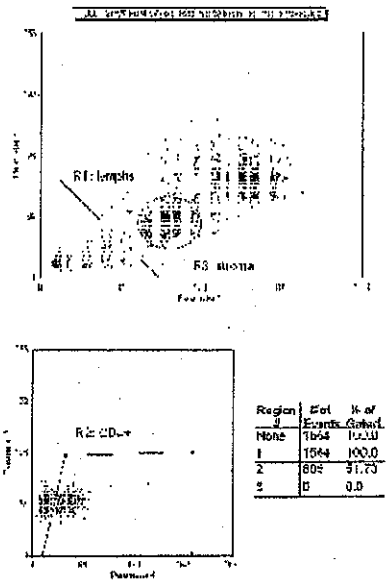


Figure 12: The three plots above are examples of a manual preparation of the Erythrolyse/Stabilyse system. The left and center plot is a sample analyzed with the PointCare optics and the right is using the Drew optics. The center plot demonstrates the increased cluster presentation when using integration. For this reason, it may be necessary to add integration or increase laser power on the Drew system for increased signal.

Additional information including testing procedures and results can be found in *Drew Scientific Visit Report 010506, Drew Scientific Visit Report 021006,* and *PCT 1040, pages 16-19.*

iv. The Excell 22 is currently going through a revision to replace obsolete electronics that may have been a concern to PointCare for future manufacturability.

The need to add the PointCare lyse reagents and gold delivery module present the need for I/O ports in the Excell 22. These ports are available for implementation of the CD4 assay fluid handling.

There is a new power supply design that will meet the PointCare business and marketing needs. A power budget of the Excell 22 is acceptable so that in case of a power failure, a sample may be completed and the system may

safely be shutdown with the presence of an Uninterruptible Power Supply (UPS). The possible use of an automobile battery pair for daily operation is still to be determined.

The data collection electronics only have peak detection (no integral channel). There is a benefit to adding integration due to increased CD4 cluster presentation. This will have to be explored further.

An onboard processor and touch screen monitor would be desirable for a future revision, but an external touch screen should be acceptable at this time.

v. The immunogold dispensing does not have to be done at a high precision (only ~10%). Syringes for immunogold and accelerant will have to be added to the Excell 22 for CD4 analysis. Because of the small volumes used, it may be necessary to add a pipetting system for the gold and accelerant reagents. The pipetting mechanism would also be necessary for the dried gold reconstitution. Attention to fluid line lengths and internal diameters are needed to ensure minimal loss of gold reagent to waste. In-line mixing may be needed for the blood, gold, and accelerant mixture.

A temperature control module for the bulk gold reagent will need to be added to the Excell 22. The bulk reagent temperature specifications have not yet been determined, but it is expected to be 2-25° C.

vi. The gates and regions for analytical software have been established for CD4 analysis. The lymphocyte gate can be placed in the low angle vs. wide angle scatter parameters. After gating on lymphocytes, the CD4 cluster can be seen using extinction vs. a modified super-wide angle (right-angle scatter). Because analysis will be dual platform, a conservative (small CV) gate can be chosen for purity of lymphocytes to obtain a CD4%. This can then be applied to the lymph count obtained by either the impedance channel or the gold-free lymph count from the cytometer.

vii. The Excell 22 user interface (UI) will have to be modified to accommodate a CD4 testing option, as well as a hematology only test. The UI will also need to be modified for CD4 and external controls.

viii. Currently, the Excell 22 has open cuvettes that may allow dust to enter the mixing chamber. A cover will have to be developed to prevent contamination to mixing chambers.

ix. The auto-sampler in existence for the Excell 22 can operate uninterruptible for 30 samples at a time. This is an expected time of 90 minutes of automated operation for a CD4 test. There is a barcode reader for positive sample identification. No testing has been done, but modification for a CD4 assay appears favorable.

x. Internal control points may have to be implemented. Currently, door

and cover sensors, reagent level sensing, database verification, and volume check (auto-sampler only) exist, but some anticipated hardware controls for temperature and humidity are to be introduced later. There are existing flags for hematology parameters but new flagging criteria and control points for CD4 analysis will have to be included as well.

i. Other Test Results

n/a

4. Discussion

j. The integration may be necessary to overcome differences in CD4 cluster presentation. Patient to patient variability can be as much as 30% due to number of CD4 antigen sites. A study done on normal patients by Bikoue et al. found that the average number of CD4 antigen sites on a T-Lymphocyte is 47,000 ± 14,000 (±30%). The difference in number of CD4 antigen sites could present differences in CD4 cluster presentation. A patient with a low number of antigen sites could have large overlap between CD4- and CD4+ which would be difficult to resolve.

The integration also would decrease noise on the RAS channel. The CD4 absolute count is less than 50 counts/μL in many patients who are in late stage AIDS. This is in the range of noise on the RAS detector when using peak detection only. By adding integration, the low CD4 counts should be easier to detect.

5. Conclusions

k. The Excell 22 system can be modified to accommodate a CD4 immunogold assay. The Excell 22 can be adapted to meet the needs of a developing world market for high volume CD4 analysis.

6. Recommendations

l. The Erythrolyse II/Stabilyse lysing system should be used to obtain a CD4 cluster on the Excell 22. The Erythrolyse II produced a larger separation between the CD4- and CD4+ clusters than the Drew lyse.

m. The mixing sequence with the Drew Excell 22 paddle mixer needs to be optimized for the mixing chamber to be used. This can be developed using the mixer breadboard and an AuRICA instrument and compared to vortexing as a standard.

n. The Excell 22 optics can be used for the CD4 immunogold assay with the following modifications:

i. An additional PMT now should be placed on the opposite side of the " super-wide angle" detector to act as a "right-angle scatter" detector. There should be no mask on this side and testing will need to be done to determine the need for a light collection lens.

ii. The interior of the optical assembly should have a black finish.

iii. A power increase or change to the current Excell 22 laser may be necessary. This should be pursued as part of assay and system optimization when rapid sample delivery is available. Options for integration on the RAS detector will also have to be examined. The beam size appears to be appropriate for CD4 analysis.

o. Create new module for handling PointCare lyse reagents and gold delivery module. Data handling for the additional detector must be addressed as well.

p. A bulk gold reagent must be developed for this system. Number of uses, reagent drying method, reconstitution method, and temperature control must be developed at PointCare.

q. Gating strategies for the CD4 cluster need to be developed for the Excell 22. Only a CD4% will be necessary for this part of the analysis. There currently is no integration for scatter parameters in the Excell 22. This will create more globular cell clusters (CD4- and CD4+) and may be more easily analyzable by histogram analysis methods.

r. Modifications to the Drew UI must be done to accommodate CD4 whole bloods and controls.

s. Dust covers for open cuvettes should be designed to prevent particulate interference.

t. The auto-sampler sequence will have to be modified to allow sampling for CD4 analysis.

u. Internal control points for hardware and flagging criteria for CD4 analysis must be

implemented.

Dear Roger,

Our meetings here with Rich, Harry, and Frank really moved right along, and I think everyone is on the same page.

I have attached Don Barry's report regarding the system work that you, Romiya, and he did in Dallas. I think that there is one very important new conclusion which you can read on Page 14 and 16. I can summarize it here:

Make no change in laser power from the current Excell 22 configuration. The advantage is of course that no current hematology analysis will need to be changed in the new system. However, even though with the current laser power there are two clusters, there really is insufficient CD4 cluster separation. If, however one was to add an integrator to the new PMT electronics, the cluster separation should be fine.

Here is why we say the cluster separation is "insufficient". The problem with CD4 analysis is that there is a 30% CV in the mean number of CD4 receptors on lymphocytes from patient to patient. It has nothing to do with HIV or the stage of the diasease. This means that the CD4 positive cluster position on the right angle scatter axis moves plus and minus about 50% if you take into account extreme cases. For this reason, you need a pretty big valley between populations when you are looking at the "average" patient in order to deal with the extreme low antigen density patients.

If you look at Don's dot plots on page 14, you will see the following illustration: The left-hand plot is the PointCare optics and peak detection for the signals. The right-hand plot is the Drew (new) optics and also peak detection for the signals. The plots are similar inasmuch as there is not a wide valley between the two clusters. The middle plot is the same sample and same run as the left-hand plot with PointCare optics, but analyzed through an integrator (we get both peak and integral outputs on PointCare). You can see the dramatic improvement in the size of the valley with the integrator.

Don and I propose that we include an integrator on the new PMT output. We have had a lot of experience with flow cytometry integrators, and in fact we have an excellent contractor near here that builds them for us. I am sure that he could design and build the appropriate board for you very quickly.

Let either Don or me know if there are any changes or additions that you would like to make to the report.

Thanks, and we are looking forward to seeing you in Boston.

Peter